



RESEARCH ARTICLE

REVISÉ Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with childhood pneumonia in Kilifi, Kenya [version 2; peer review: 2 approved]

Previously titled: Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya

Grievan P. Otieno ¹, Nickson Murunga ¹, Charles N. Agoti ¹, Katherine E. Gallagher^{1,2}, Juliet O. Awori¹, D. James Nokes ^{1,3}

¹Epidemiology and Demography Department, Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya

²London School of Hygiene and Tropical Medicine, London, UK

³School of Life Sciences and Zeeman Institute for Systems Biology and Infectious Disease Epidemiology Research (SBIDER), University of Warwick, Coventry, UK

V2 First published: 26 Jun 2020, 5:150
<https://doi.org/10.12688/wellcomeopenres.16037.1>

Latest published: 22 Sep 2020, 5:150
<https://doi.org/10.12688/wellcomeopenres.16037.2>

Abstract

Introduction: Human coronaviruses (HCoVs) circulate endemically in human populations, often with seasonal variation. We describe the long-term patterns of paediatric disease associated with three of these viruses, HCoV-NL63, OC43 and 229E, in coastal Kenya.



Methods: Continuous surveillance of pneumonia admissions was conducted at the Kilifi county hospital (KCH) located in the northern coastal region of Kenya. Children aged <5 years admitted to KCH with clinically defined syndromic severe or very severe pneumonia were recruited. Respiratory samples were taken and tested for 15 virus targets, using real-time polymerase chain reaction. Unadjusted odds ratios were used to estimate the association between demographic and clinical characteristics and HCoV positivity.


Results: From 2007 to 2019, we observed 11,445 pneumonia admissions, of which 314 (3.9%) tested positive for at least one of the HCoV types surveyed in the study. There were 129 (41.1%) OC43, 99 (31.5%) 229E, 74 (23.6%) NL63 positive cases and 12 (3.8%) cases of HCoV to HCoV coinfection. Among HCoV positive cases, 47% (n=147) were coinfecting with other respiratory virus pathogens. The majority of HCoV cases were among children aged <1 year (66%, n=208), though there was no change in the proportion infected by age. HCoV-OC43 was predominant of the three HCoV types throughout the surveillance period. Evidence for seasonality was not identified.


Conclusions: Overall, 4% of paediatric pneumonia admissions were associated with three endemic HCoVs, with a high proportion of cases co-occurring with another respiratory virus, no clear seasonal pattern,

Open Peer Review

Reviewer Status  

	Invited Reviewers	
	1	2
version 2		
(revision)		
22 Sep 2020	report	report
	↑	↑
version 1		
26 Jun 2020	report	report

1. **David P. Moore** , University of the Witwatersrand, Johannesburg, South Africa
 University of the Witwatersrand,
 Johannesburg, South Africa

2. **Sema Nickbakhsh** , University of Glasgow, Glasgow, UK

Any reports and responses or comments on the article can be found at the end of the article.

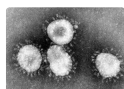
and with the age-distribution of cases following that of pneumonia admissions (i.e. highest in infants). These observations suggest, at most, a small severe disease contribution of endemic HCoVs in this tropical setting and offer insight into their potential future burden and epidemiological characteristics.

Keywords

Human coronavirus, NL63, OC43, 229E



This article is included in the [KEMRI | Wellcome Trust](#) gateway.



This article is included in the [Coronavirus \(COVID-19\)](#) collection.

Corresponding author: Grieben P. Otieno (grievenoti@gmail.com)

Author roles: **Otieno GP:** Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Murunga N:** Data Curation, Formal Analysis, Investigation, Validation, Visualization, Writing – Review & Editing; **Agoti CN:** Investigation, Validation, Writing – Review & Editing; **Gallagher KE:** Investigation, Validation, Writing – Review & Editing; **Awori JO:** Investigation, Validation, Writing – Review & Editing; **Nokes DJ:** Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The study was supported by the Wellcome Trust (102975; 220985), and PERCH (48968 from The Bill & Melinda Gates Foundation to the International Vaccine Access Center, Department of International Health, Johns Hopkins Bloomberg School of Public Health).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2020 Otieno GP *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Otieno GP, Murunga N, Agoti CN *et al.* **Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with childhood pneumonia in Kilifi, Kenya [version 2; peer review: 2 approved]** Wellcome Open Research 2020, 5:150 <https://doi.org/10.12688/wellcomeopenres.16037.2>

First published: 26 Jun 2020, 5:150 <https://doi.org/10.12688/wellcomeopenres.16037.1>

REVISED Amendments from Version 1

Revisions have been made to the manuscript based on recommendations from the reviewers. The following are the major changes:

1. In the methods section, we have highlighted the various sample collection methods that were used over time throughout the study period and further gave a brief discussion regarding the implication these changes could have on virus detection.
2. In the results section, we have added a column of odds ratios for any of the three HCoV types that we tested
3. In the discussion section, we have added text regarding the following:

We look at possible explanations on the observed variations of HCoV prevalence across age bands by exploring the linkage between potential community incidence and the likelihood for hospitalisation.

We highlight in the limitations that HKU1 results were not included in this study because they were available for a short period (24 months) when PERCH was conducted compared to the entire period of our study (13 years).

We further discuss the potential under-estimation of HCoV prevalence based on our eligibility criteria which did not include other clinical features such as fever.

Any further responses from the reviewers can be found at the end of the article

Introduction

To date, seven human coronaviruses (HCoVs) have been identified, of which four (NL63, HKU1, OC43 and 229E) are known to be endemic among humans¹⁻⁴. Endemic HCoV types are detected in a small but non-negligible proportion of respiratory tract infections; mild cases occur across a wide age-range and severe disease is predominant in young children and the elderly⁵⁻⁸. A further three HCoVs have emerged in recent years and caused epidemics: SARS-CoV the agent of severe acute respiratory syndrome in China⁹, MERS-CoV the cause of Middle East respiratory syndrome in the Middle-East¹⁰ and most recently SARS-CoV-2, the aetiological agent of the current pandemic of coronavirus disease 2019 (COVID-19)¹¹. To date, there are limited preventive options against HCoV infections and no effective anti-viral treatment¹². Understanding the epidemiology of HCoVs can play a critical role in prediction, prevention and control of HCoV infection. In addition, data on endemic HCoVs may inform expectations for SARS-CoV-2 if it becomes endemic. Our study aims to describe the circulation patterns of three endemic HCoVs (OC43, 229E, and NL63) over time using data from a long-term surveillance programme in a rural coastal setting in Kenya, 3° south of the equator.

Methods

Study setting

A prospective study was established in 2007 for long-term continuous respiratory virus surveillance among pneumonia admissions to Kilifi County Hospital (KCH)⁶ in order to develop improved epidemiological understanding, estimate disease

burden and provide suitable baseline data for future vaccine studies. KCH is the referral hospital within the Kilifi Health and Demographic Surveillance System (KHDSS), in the northern coastal region of Kenya¹³. The location experiences two rainy seasons approximately from April to July and October to December, with median maximum temperature of 33°C (IQR: 31–36), median minimum temperature of 23°C (IQR: 22–24), and median relative humidity of 78% (IQR: 71–87) (unpublished weather station data). Children aged 1 day to 59 months admitted to KCH with clinical symptoms of severe or very severe pneumonia were recruited. Written informed consent was sought from parents/guardians of the children prior to sample collection. In this paper we define severe pneumonia as history of cough or difficulty breathing and chest indrawing while very severe pneumonia is defined as history of cough or difficult breathing and at least one of inability to feed, prostration, unconsciousness, or oxygen saturation of <90% by fingertip pulse oximetry. We use the term pneumonia to refer to all cases of clinically severe or very severe pneumonia¹⁴. The variables extracted from the hospital surveillance database include; Demographic characteristics (sex, KHDSS residency status, age), presence/absence of clinical features (history of cough, difficulty breathing, cyanosis, nasal flaring, chest indrawing, crackles, wheeze, inability to drink, vomits everything, fever defined as axillary temperature $\geq 37.5^\circ\text{C}$, oxygen saturation levels, conscious level: agitated, lethargic, prostration or unconscious, pneumonia status: severe or very severe), laboratory test results, and hospitalisation outcomes (admission to the high dependency unit, discharge outcomes: alive or dead)

Laboratory methods

Specimens collected between January 2007 and December 2019 were processed and screened for three HCoVs (OC43, NL63 and 229E) and at least 12 other respiratory viral pathogens (RSV (A and B), rhinovirus, HCoVs (NL63, OC43, 229E), influenza (A, B and C), parainfluenza virus (1–4), adenovirus, and human metapneumovirus) using real-time polymerase chain reaction (RT-PCR). Sample testing was initially performed in 2007 using the LightCycler Fast Start DNA MasterPLUS HybProbe kit (Roche)⁶, then multiplex RT-PCR using Qiagen Quantifast multiplex RT-PCR kit (Qiagen, United Kingdom) in triplex sets on an ABI 7500 system, from January 2007 until the present day^{15,16}; additionally, a proportion of samples were tested using a 33-pathogen multiplex quantitative PCR (FTD Resp-33, Fast Track Diagnostics, Sliema, Malta) as part of the multi-country PERCH study¹⁷, between August 2011 and December 2013. A variety of collection methods was used: nasal wash (2007 to 2009), nasopharyngeal flocked swab (2010 to 2014), or combined nasopharyngeal swab and oropharyngeal swab (2015 onwards).

Data analysis

Data analysis was done using STATA version 13.0 (Stata Corp, College Station Texas, USA). Summary statistics (counts, proportions, measures of central tendency and variation) are presented for continuous and categorical data as appropriate. We estimated unadjusted odds ratios to measure the association between demographic and clinical characteristics of the study participants and testing positive for HCoV. Three Poisson

regression models, one for each HCoV type, were used to investigate the presence of seasonality. In the models a trend variable was included and residuals plotted against month. Identification of a strong pattern by visual inspection of the residual plots would suggest presence of seasonality. The chi-square test of proportional trends was used to test for a linear trend in the proportions of samples tested or not tested for HCoV over time. To check for an association between categorical variables the chi-square test of association or Fisher's exact test was used as appropriate. The analysis code is provided as *Extended data*¹⁸.

Ethical approval

This study was approved by the Kenya Medical Research Institute Scientific Ethics Review Unit (Approval number: KEMRI/SERU/CGMR-C/027/3178).

Results

Characteristics of patients infected with HCoVs

During the 13 years of surveillance, there were 49,409 paediatric admissions of children aged 0–59 months at KCH. A total of 11,445 (23.2%) admissions were due to severe ($n=7808$, 68%) and very severe ($n=3637$, 32%) pneumonia. Out of the eligible cases, 69.5% ($n=7957$) were tested for the three HCoVs while the remainder were not tested due to refusal of consent (13.8%), discharge (13%) or death (3.7%) prior to sample collection. Cases untested did not differ from those tested in age distribution or sex ratio, but were more likely to be very severe (40.0% versus 28.3%, Fishers exact P-value <0.001).

Of the 7957 children tested, 5312 (66.7%) were aged <1 year, 1454 (18.3%) were aged 12–23 months, 620 (7.8%) were aged 24–35 months and 571 (7.2%) were in the 36–59 months age band. The proportion of tested individuals with fever (axillary temperature $\geq 37.5^\circ\text{C}$), cough and difficulty breathing was 58.3%, 83.2%, 92.6%, respectively. A total of 314 (3.9%) tested positive for at least one of the three HCoV targets. Among the HCoV positives, 129 (41.1%) had OC43, 99 (31.5%) had 229E, 74 (23.6%) had NL63 and 12 (3.8%) had coinfections between the three HCoV types. Among all the samples tested, the overall prevalence of NL63 was 1% ($n=80$), 1.7% for OC43 ($n=137$) and 1.4% for 229E ($n=109$).

The characteristics of the patients positive for any and for each HCoV type or infection combination are described in [Table 1](#). HCoV positive cases were predominantly children aged <1 year (66.2%) and those aged 12–23 months (18.2%). The burden of infection with at least one HCoV, among all pneumonia admissions, was highest in infants and decreased with increasing age (2.6% for those under 1 year and 0.7% for 12–23 months). The same pattern was seen for each individual HCoV type (not shown). However, the proportion of samples testing positive for HCoV (3.9%) did not vary with age group (Fisher's exact p-value = 0.753). Among all HCoV positive participants, mean age was 11 months (median 7 months), there were fewer females than males and fewer with

very severe compared to severe pneumonia. At least half of the HCoV positives presented with fever (58%) and nasal flaring (53%).

Clinical outcomes of HCoV-infected patients

Over a quarter of those positive for at least one of the three endemic HCoVs investigated in this study were admitted to the high dependency unit and of those positive for OC43, 13.5% ($n=10$) died while 7.8% ($n=10$) and 7.1% ($n=7$) died of those positive for NL63 and 229E, respectively. A large proportion of these deaths were observed among those with underlying co-morbidities ([Figure 1](#)). None of the HCoVs were statistically significantly associated with any of the specific clinical signs or outcomes investigated (p-values >0.05) except death among NL63 cases ([Table 2](#)); however, we had limited power to detect associations given the small number of HCoV positive cases.

Co-infection with other respiratory viruses

About 47% ($n=147$) of the 314 HCoV cases were co-infected with other viral respiratory pathogens; respiratory syncytial virus (RSV) and human rhinovirus (HRV) jointly accounted for >50% of all HCoV coinfections with other pathogens. A similar coinfection pattern was observed for each HCoV tested ([Figure 2](#)). Throughout the surveillance period, there were three cases (one of NL63 and two of OC43) aged <1 year that were readmitted and tested positive for the same HCoV as the first admission. The NL63 readmission occurred 10 days after discharge from the first admission while the OC43 readmissions were at 3 and 21 days after discharge from the first admission. The NL63 case had a discharge diagnosis of neonatal sepsis for the first admission and gastroenteritis plus lower respiratory tract infection (LRTI) for the readmission. One of the OC43 cases had a discharge diagnosis of LRTI for both admissions while the other had immunosuppression plus malnutrition in the first and immunosuppression plus septicemia for the second admission.

Temporal patterns of different HCoVs

NL63 and OC43 were observed fairly consistently throughout the surveillance period while fewer cases of HCoV-229E were observed from the middle of 2011 and were not detected subsequent to 2016 ([Figure 3](#)). The highest numbers of cases were observed in the periods April to June for NL63, June to September for OC43 and January to March for 229E. Pooling data for all HCoVs, there were more cases in the colder months (May to September) than the hotter months (October to April) ([Figure 4](#)), as for OC43, but NL63 was more common in the first half of the year, and 229E in the second half of the year. However, time series models did not indicate a seasonal pattern for any of the HCoVs ([Figure 5](#)) over the years. The proportion of samples tested for HCoV did not change over time among those with severe pneumonia ($\chi^2_{(1)}=3.11$; p-value = 0.078) but changed among those with very severe pneumonia $\chi^2_{(1)}=149.11$; p-value < 0.001).

De-identified raw data for this study are available as *Underlying data*¹⁸.

Table 1. Demographic and clinical characteristics of study children (aged under 5 years) admitted with severe or very severe pneumonia to Kilifi County Hospital, Kilifi, Kenya 2007–2019, by HCoV type (n=314).

Variable		NL63 (n=74)	OC43 (n=129)	229E (n=99)	HCoVs coinfections (n=12)	Total (n=314)
		n (%)	n (%)	n (%)	n (%)	n (%)
Age (months)	Median (IQR)	8 (3-20)	7 (2-15)	8 (2-19)	5.5 (2-8.5)	7 (2-16)
Age categories	0–11 months	49 (66.2)	87 (67.4)	61 (61.6)	12 (91.7)	208 (66.2)
	12–23 months	11 (14.9)	24 (18.6)	22 (22.2)	0 (0.00)	57 (18.2)
	24–35 months	11 (14.9)	8 (6.2)	9 (9.1)	1 (8.3)	29 (9.2)
	36–59 months	3 (4.0)	10 (7.8)	7 (7.1)	0 (0.00)	20 (6.4)
Sex	Female	22 (29.7)	60 (46.5)	36 (36.4)	6 (50.0)	124 (39.5)
	Male	52 (70.2)	69 (53.5)	63 (63.6)	6 (50.0)	190 (60.5)
Pneumonia status	Severe	49 (66.2)	101 (78.3)	76 (76.8)	10 (83.3)	236 (75.2)
	Very severe	25 (33.8)	28 (21.7)	23 (23.2)	2 (16.7)	78 (24.8)
Clinical presentation	Cough	55 (74.3)	112 (86.8)	85 (85.9)	12 (100.00)	264 (84.1)
	Difficulty breathing	67 (90.5)	118 (91.5)	94 (95.0)	10 (83.3)	289 (92.1)
	Fever*	43 (58.1)	75 (58.1)	54 (54.6)	10 (83.3)	182 (58.0)
	Prostration or unconscious	17 (23.0)	18 (14.0)	14 (14.1)	1 (8.3)	50 (15.9)
	Chest Indrawing	65 (87.8)	122 (94.6)	89 (89.9)	12 (100.00)	288 (91.7)
	Wheeze	7 (9.5)	16 (12.5)	18 (18.2)	0 (0.0)	41 (13.1)
	Crackle	27 (36.5)	44 (34.1)	40 (40.4)	3 (25.0)	114 (36.3)
	Nasal flaring	40 (54.1)	66 (51.2)	54 (54.6)	6 (50.0)	166 (52.9)
	Shock*	12 (16.2)	10 (7.8)	6 (6.1)	0 (0.00)	28 (8.9)
	Hypoxemia (O ₂ <90%)	12 (16.2)	14 (10.9)	13 (13.1)	1 (8.3)	40 (12.7)
	Cyanosis	1 (1.4)	1 (0.8)	1 (1.0)	0 (0.00)	3 (1.0)
	Inability to drink/feed	5 (6.8)	11 (8.5)	7 (7.1)	0 (0.00)	23 (7.3)
	Vomits everything	7 (9.5)	6 (4.7)	0 (0.00)	0 (0.00)	13 (4.14)
	Duration of hospital stay					
Outcomes	Mean (SD)	6.3 (6.8)	5.7 (6.1)	5.2 (5.5)	3.8 (2.3)	5.6 (6.0)
	Median (IQR)	4 (3-6)	3 (2-7)	3 (2-6)	6.5 (3-10)	4 (2-6)
Outcomes	HDU*	27 (36.5)	31 (24.0)	22 (22.2)	2 (16.7)	82 (26.1)
	Died	10 (13.5)	10 (7.8)	7 (7.1)	0 (0.00)	27 (8.6)

* Fever is defined as axillary temperature $\geq 37.5^{\circ}\text{C}$, shock is defined as capillary refill of >3 seconds, temperature gradient or weak pulse volume. HDU indicates study participants who were critically ill and were transferred from the general ward to the high dependency unit.

** The high proportions of cough, difficulty breathing, chest indrawing, hypoxemia, prostration or unconscious and inability to drink/feed among HCoV positive cases should be interpreted with caution because these clinical signs form part of our study's eligibility criteria.

Discussion

We have described the circulation patterns of three endemic HCoVs (NL63, OC43 and 229E) in a long-term surveillance study of childhood pneumonia hospitalisations in coastal Kenya. We observed a small proportion of pneumonia admissions positive for one or more HCoVs (3.9%). While 65% of HCoV

infections occurred in children in their first year of life (either cumulatively for all HCoVs or for each individual HCoV type), this reflected the age-distribution of pneumonia admissions to the ward. Hence, contrary to other reports¹⁹, HCoV did not differ by age and this might reflect no variation in age-specific community incidence of infection in children under five, together

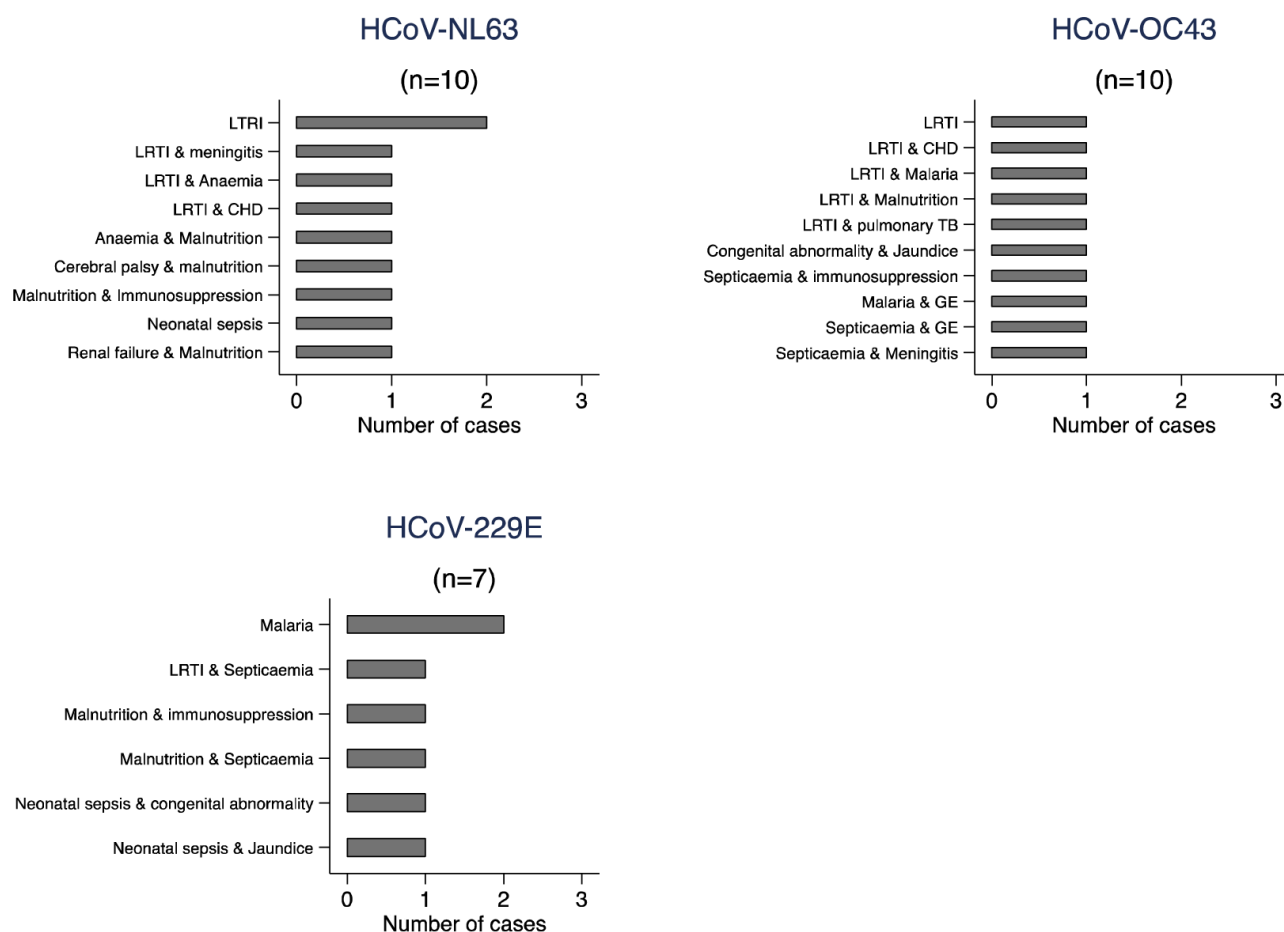


Figure 1. Frequency distribution of discharge diagnosis for mortality cases admitted to Kilifi County Hospital, Kilifi, Kenya 2007–2019, by HCoV type. LRTI=lower respiratory tract infection, CHD=congenital heart disease, TB=Tuberculosis, GE=Gastroenteritis.

with lack of variation in disease severity (likelihood of hospitalisation) across ages, or (ii) variation in age-specific community incidence by age but with a disproportionate probability of hospitalisation. Our reported prevalence is equivalent to that from a long-term hospital surveillance of seasonal coronaviruses in Scotland (4%)⁸ but lower compared to a multi-centre study across Kenya for the same three HCoVs (26/417 6.2% versus 314/7643 3.9%; $\chi^2_{(1)} = 4.43$, p-value=0.035)⁷. This latter study included locations with a wide range of climate conditions that might influence prevalence; however, the study was not big enough to stratify by location.

We did not observe seasonal variation of HCoVs compared to some other respiratory viral pathogens like RSV, as previously reported from our site¹⁴. In addition, neither peak months for pneumonia admissions nor the long rainy periods (April to July) in Kilifi were associated with HCoV peaks. This is in contrast to data from temperate settings where seasonality of HCoVs has been reported^{8,20–22}, with increased occurrence during the colder winter months. The HCoVs we have studied are known to continuously circulate among humans¹, although

in Kilifi we observed low numbers for all types. Of interest is that pneumonia associated with 229E admissions was not detected in the later years of the surveillance. We attempted to investigate if this was due to primers or probe mismatches. For all the three tested endemic CoVs we did not observe significant mismatches on the primer/probe pairs against data available from GenBank database although this investigation suffered a limitation of few sequences available globally in recent years (2015–2019) and none from East Africa. With the highest numbers and consistent presence compared to NL63 and 229E, our results suggest that OC43 is the predominant HCoV type in the coastal region of Kenya.

The present study did not have a control group by which to assess an aetiological association between the HCoVs and pneumonia. In the PERCH multi-country case-control study¹⁷ HCoVs contributed less than 1% of the etiological fraction. In our study the contribution to disease is not known (except for the relatively small set of samples from 2011–13 that were part of the PERCH study), but it is of note that around 50% co-occurred with another respiratory virus (most commonly with

Table 2. Unadjusted Odds Ratios (ORs) for demographic and clinical characteristics and the hospitalisation outcomes for children (under 5 years) admitted with severe or very severe pneumonia to Kilifi County Hospital, Kilifi, Kenya 2007-2019 by the 3 HCoV types.

		NL63		OC43		229E		Any HCoV	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	0-11 months	Ref		Ref		Ref		Ref	
	12-23 months	0.74 (0.39-1.42)	0.369	0.92 (0.59-1.45)	0.723	1.15 (0.71-1.86)	0.569	1.00 (0.74 – 1.35)	0.994
	24-35 months	1.92 (1.02-3.61)	0.043	0.72 (0.35-1.48)	0.371	1.23 (0.63-2.39)	0.547	1.20 (0.81 – 1.79)	0.360
	36-59 months	0.51 (0.16-1.65)	0.263	0.98 (0.51-1.89)	0.949	0.93 (0.43-2.03)	0.854	0.89 (0.56 – 1.42)	0.627
Sex	Female	Ref		Ref		Ref		Ref	
	Male	1.62 (1.01-2.60)	0.046	0.81 (0.58-1.14)	0.221	1.27 (0.86-1.88)	0.233	1.13 (0.89 – 1.42)	0.302
Cough	No	Ref		Ref		Ref		Ref	
	Yes	0.64 (0.38-1.08)	0.098	1.43 (0.86-2.39)	0.168	1.37 (0.78-2.42)	0.268	1.07 (0.79 – 1.46)	0.671
Breathing difficulty	No	Ref		Ref		Ref		Ref	
	Yes	0.63 (0.31-1.27)	0.196	0.83 (0.46-1.52)	0.544	1.38 (0.61-3.17)	0.440	0.93 (0.61 – 1.41)	0.720
Fever*	No	Ref		Ref		Ref		Ref	
	Yes	1.13 (0.72-1.78)	0.587	1.04 (0.73-1.46)	0.839	0.94 (0.65-1.38)	0.766	0.99 (0.79 – 1.24)	0.908
Prostration/unconsciousness	No	Ref		Ref		Ref		Ref	
	Yes	1.44 (0.83-2.46)	0.189	0.85 (0.52-1.39)	0.517	0.84 (0.49-1.46)	0.543	1.00 (0.74 – 1.37)	0.982
Chest indrawing	No	Ref		Ref		Ref		Ref	
	Yes	0.68 (0.33-1.37)	0.279	1.62 (0.75-3.48)	0.217	0.86 (0.44-1.65)	0.64	0.96 (0.64 – 1.44)	0.835
Inability to feed	No	Ref		Ref		Ref		Ref	
	Yes	0.54 (0.22-1.35)	0.189	0.71 (0.38-1.32)	0.283	0.62 (0.29-1.34)	0.226	0.66 (0.43 – 1.02)	0.061
Hypoxemia (O ₂ <90%)	No	Ref		Ref		Ref		Ref	
	Yes	0.92 (0.51-1.67)	0.780	0.58 (0.34-0.99)	0.046	0.64 (0.34-1.14)	0.131	0.68 (0.49 – 0.96)	0.026
Pneumonia status	Severe	Ref		Ref		Ref		Ref	
	Very severe	1.22 (0.76-1.95)	0.406	0.71 (0.47-1.06)	0.094	0.71 (0.45-1.12)	0.143	0.83 (0.64 – 1.08)	0.162
Hospital stay	≤ 4 days	Ref		Ref		Ref		Ref	
	> 4 days	1.06 (0.68-1.65)	0.807	0.85 (0.60-1.21)	0.373	0.72 (0.49-1.08)	0.110	0.88 (0.70 – 1.11)	0.285
Death	No	Ref		Ref		Ref		Ref	
	Yes	1.98 (1.02-3.87)	0.045	1.07 (0.56-2.05)	0.842	0.93 (0.43-2.03)	0.872	1.29 (0.86 – 1.93)	0.218

*Fever is defined as axillary temperature ≥ 37.5° C

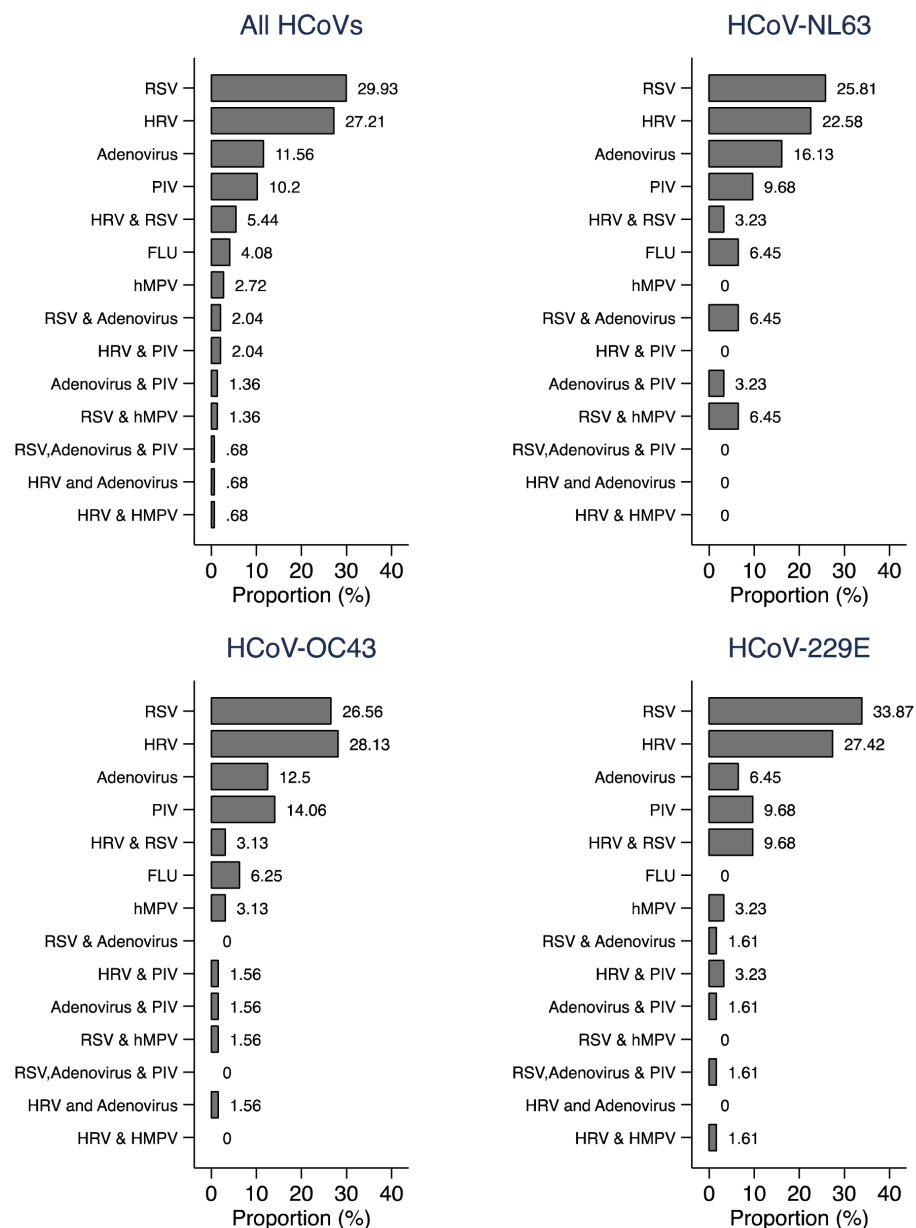


Figure 2. Percentage distribution of coinfections between HCoVs and other viral pathogens for pneumonia cases admitted to Kilifi County Hospital, Kilifi, Kenya 2007–2019. RSV=respiratory syncytial virus (A and B), HRV=human rhinovirus, PIV=parainfluenza, FLU=influenza (A, B and C), hMPV= human metapneumovirus.

RSV), the risk was not age-dependent, there was no clear association between any of the viruses with the any of the specific clinical signs or outcomes investigated and in 26% of deaths with a HCoV detected there was a likely alternative diagnosis to pneumonia. This suggests the presence of incidental HCoV in Kilifi and other clinical groups (hence an under-estimation in this study), though causality of death outcomes was not differentially identified in this study. While we have been able to sequence the virus from a proportion of the positive specimens^{23,24}, we cannot assume 100% specificity, and even a modest level of false positivity could account for many of the positive

diagnoses and argues for caution in interpreting the prevalence estimates. Of relevance also is that few (~1%) of the 314 children positive for at least one HCoV were subsequently HCoV-positive readmissions. While this is a crude analysis which ignores censoring at the start and end of the surveillance, and alternative hospitals where patients may have been admitted, it might be an indicator of low probability of severe reinfection. Alternatively, these readmissions might be children with a prolonged infection having not fully recovered at their first discharge. In addition, HKU1 was only tested during the 24 month period that PERCH study was conducted hence

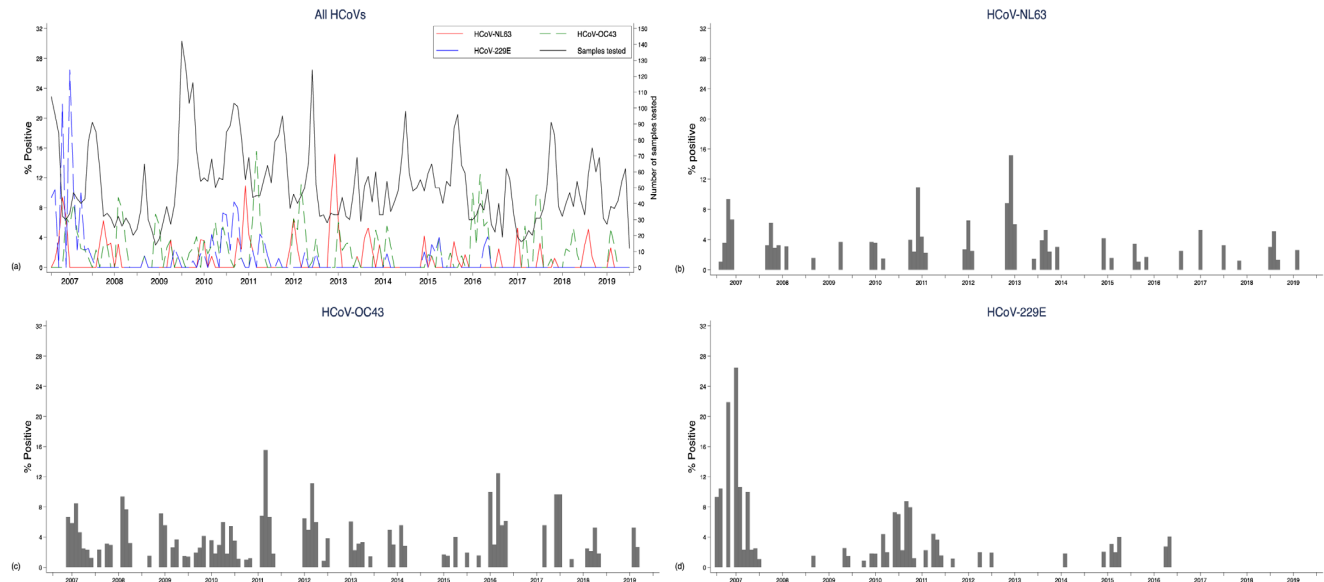


Figure 3. Monthly prevalence (%) pneumonia admissions at Kilifi County Hospital, Kilifi, Kenya 2007–2019 by HCoV type. The panel shows proportions for all HCoVs (a), NL63 (b), OC43 (c) and 229E (d).

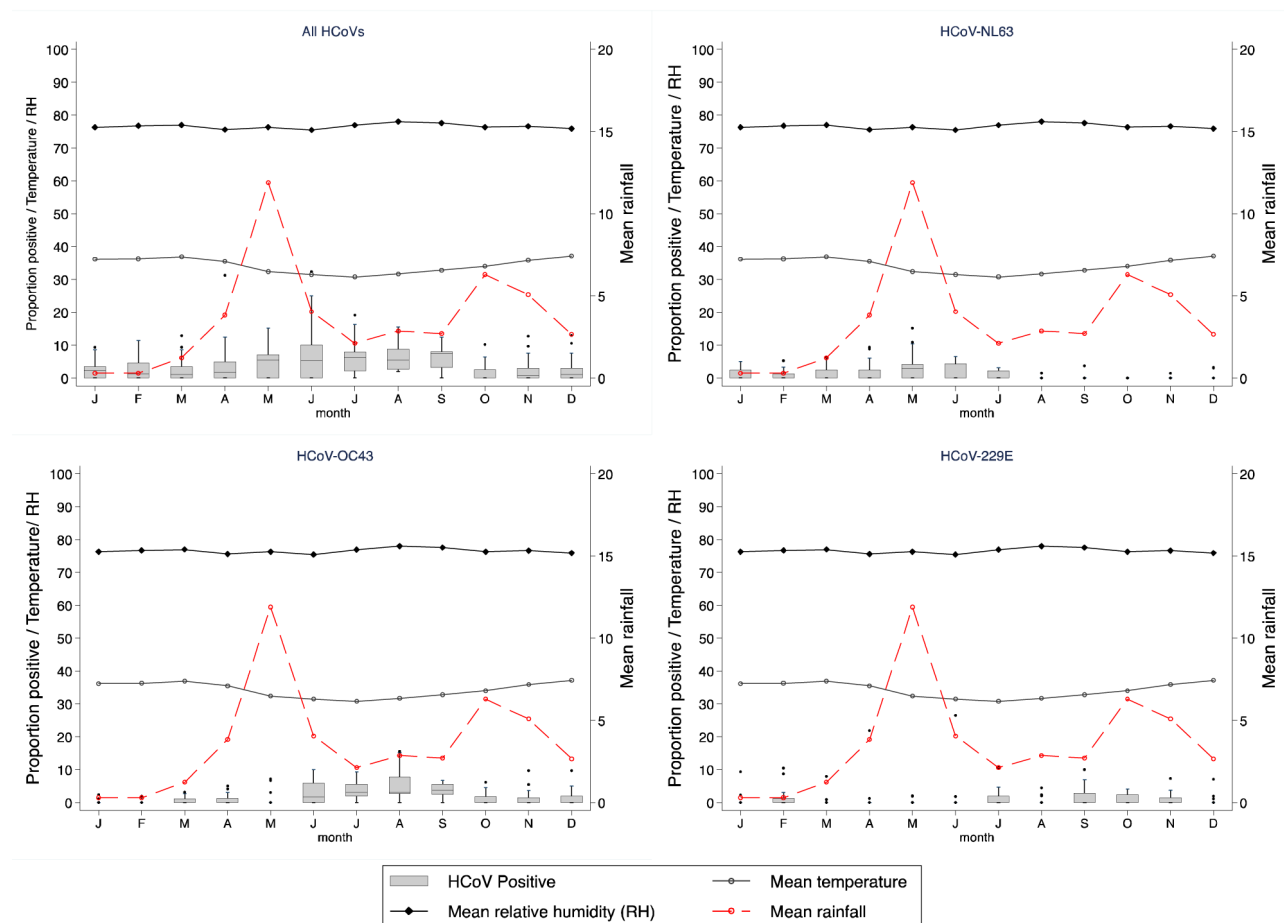


Figure 4. Proportion of monthly positive cases observed at Kilifi County Hospital, Kilifi, Kenya by HCoV type over a period of 13 years (2007–2019). The primary y-axis denotes the proportion of samples positive for HCoV the average monthly maximum temperature in °C and relative humidity (%) while the secondary y-axis denotes the average monthly rainfall in millimetres.

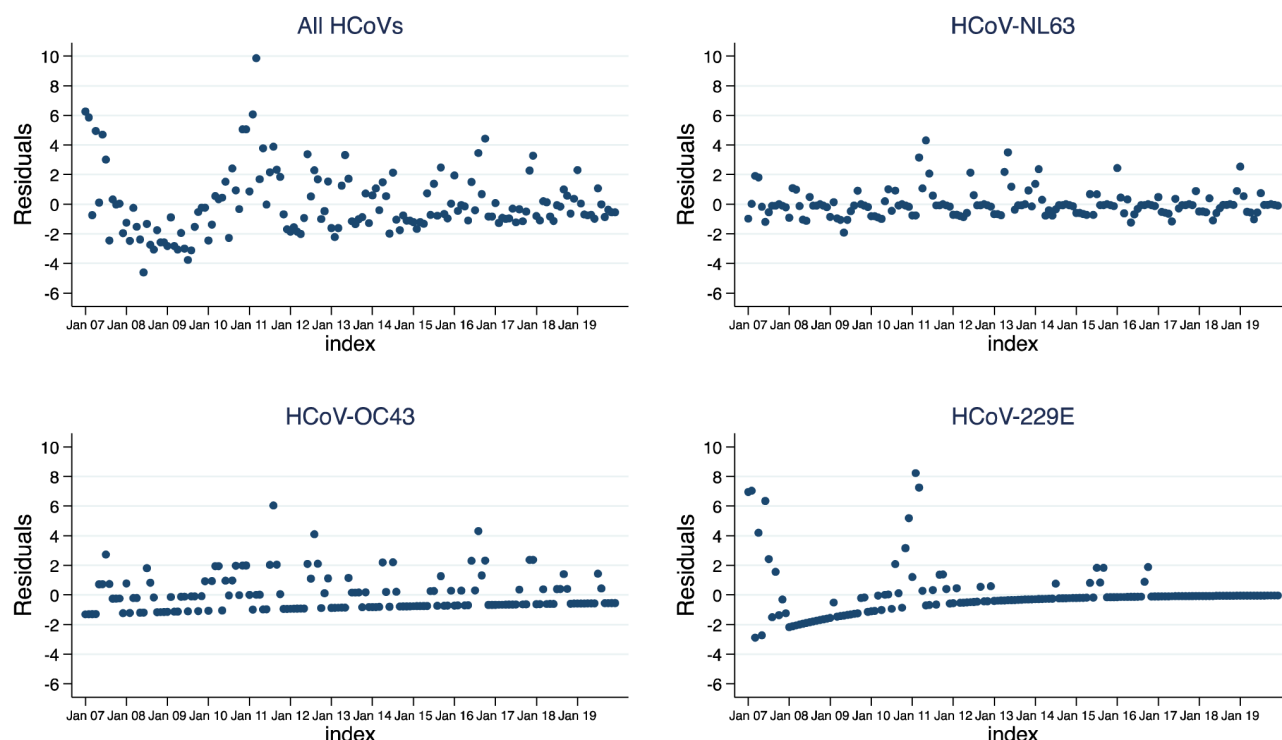


Figure 5. Plot of time series model residuals against month separated by HCoV type using pneumonia surveillance data at Kilifi County Hospital, Kilifi, Kenya 2007–2019. See *Methods* for statistical details.

samples tested for HKU1 were considered unrepresentative of the entire study period and therefore not included in this analysis.

Over the surveillance period, we have changed our sample collection and testing methods. This is a limitation; we did not conduct a sensitivity analysis to compare the different PCR methods, and the addition of an OP swab increases the number of viruses found by NP alone (by 14% for HCoVs)¹⁵. Some of the observed patterns may have been influenced by these changes. It should be noted that fever, either on history or as measured at the time of admission, was not an inclusion criterion for eligibility, which might have influenced the prevalence of HCoV. If the endemic COVs are strongly associated with symptoms of fever, as is SARS CoV-2, then we might be under-estimating the prevalence due to a selection bias. However, interestingly, only 58% of the HCoV positive cases had axillary temperature of $\geq 37.5^{\circ}\text{C}$. A further limitation is that only a fraction (70%) of pneumonia cases was tested for HCoV. We have previously shown that those untested tend to be more severely ill and less likely to be virus positive¹⁴. The proportion tested has not substantially changed over time among individuals with severe pneumonia but changed among those with very severe pneumonia. Similarly, we did not observe a difference in the ages of those tested and those untested for HCoV across time. We employed various collection methods and these are known to differ in the range of viruses detected. For example, the addition of an OP swab to an NP swab has been found to increase virus yield¹⁵.

In conclusion, in this tropical setting we find the three endemic coronaviruses were observed at low prevalence, not dissimilar to influenza and metapneumovirus, but considerably lower than for RSV and human rhinovirus. There was no clear seasonal variation. As the pandemic of COVID-19 takes its course, it is of interest to speculate whether the SARS-CoV-2 virus will become endemic and continuously co-circulate in the human population with the existing HCoVs^{7,25}. The epidemiology of endemic HCoVs can be used to inform our expectations of SARS-CoV-2 in childhood, its potential severity and inter-species interactions and competition.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya. <https://doi.org/10.7910/DVN/ZQ1DJY>¹⁸.

This project contains the following underlying data:

- KCH_paed_ARI_surv_pneumo (CSV). (De-identified underlying data for each patient in the study.)
- KCH_paed_ARI_surv_pneumo-1 (SAV). (De-identified underlying data for each patient in the study.)
- GPotieno_HCOV_Codebook (PDF). (Data dictionary and codebook.)

The data have been de-identified, and hence lack personally identifiable information. To request access to additional variables from this dataset go to 'Data Governance' on <http://kemri-wellcome.org/about-us/#ChildVerticalTab15> and submit an 'External Request' to the Data Governance Committee (dgc@kemri-wellcome.org).

Extended data

Harvard Dataverse: Replication Data for: Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya. <https://doi.org/10.7910/DVN/ZQ1DJY>¹⁸.

This project contains the following extended data:

- 1_descriptive_analysis (DO). (Scripts used to generate information in the tables, data on frequencies, HCOVs virus distributions and proportions.)
- 2_graph_outputs (DO). (Code used to generate charts in the paper.)

- 3_ORs (DO). (Code to fit univariable logistic regression models for each HCoV type.)
- 4_seasonality (DO). (scripts for analyses of HCoV type seasonality.)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgments

We thank the study participants and their parents/ guardians, clinical and research team members of the Virus Epidemiology and Control group (<http://virec-group.org/>) who were involved at various stages of this study including sample collection and processing. This paper has been approved for publication by the director of the Kenya Medical Research Institute.

References

- van der Hoek L, Pyrc K, Jebbink MF, et al.: **Identification of a new human coronavirus.** *Nat Med.* 2004; **10**(4): 368–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Woo PCY, Lau SKP, Chu CM, et al.: **Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia.** *J Virol.* 2005; **79**(2): 884–95.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tyrrell DA, Bynoe ML: **Cultivation of a Novel Type of Common-cold Virus in Organ Cultures.** *Br Med J.* 1965; **1**(5448): 1467–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hamre D, Procknow JJ: **A new virus isolated from the human respiratory tract.** *Proc Soc Exp Biol Med.* 1966; **121**(1): 190–3.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nyiro JU, Munywoki P, Kamau E, et al.: **Surveillance of respiratory viruses in the outpatient setting in rural coastal Kenya: baseline epidemiological observations [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2018; **3**: 89.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Berkley JA, Munywoki P, Ngama M, et al.: **Viral etiology of severe pneumonia among Kenyan young infants and children.** *JAMA.* 2010; **303**(20): 2051–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sipulwa LA, Ongus JR, Coldren RL, et al.: **Molecular characterization of human coronaviruses and their circulation dynamics in Kenya, 2009–2012.** *Viral J.* 2016; **13**(1): 18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nickbakhsh S, Ho A, Marques DFP, et al.: **Epidemiology of seasonal coronaviruses: Establishing the context for COVID-19 emergence.** *medRxiv.* 2020.
[Publisher Full Text](#)
- Peiris JSM, Lai ST, Poon LLM, et al.: **Coronavirus as a possible cause of severe acute respiratory syndrome.** *Lancet.* 2003; **361**(9366): 1319–25.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Raj VS, Osterhaus ADME, Fouchier RAM, et al.: **MERS: emergence of a novel human coronavirus.** *Curr Opin Virol.* 2014; **5**: 58–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- World Health Organization: **Coronavirus.** [cited 2020 Mar 20].
[Reference Source](#)
- Fehr AR, Perlman S: **Coronaviruses: An Overview of Their Replication and Pathogenesis.** *Methods Mol Biol.* 2015; **1282**: 1–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Scott JAG, Bauni E, Moisi JC, et al.: **Profile: The Kilifi Health and Demographic Surveillance System (KHDSS).** *Int J Epidemiol.* 2012; **41**(3): 650–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nokes DJ, Ngama M, Bett A, et al.: **Incidence and Severity of Respiratory Syncytial Virus Pneumonia in Rural Kenyan Children Identified through Hospital Surveillance.** *Clin Infect Dis.* 2009; **49**(9): 1341–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hammit LL, Kazungu S, Welch S, et al.: **Added Value of an Oropharyngeal Swab in Detection of Viruses in Children Hospitalized with Lower Respiratory Tract Infection.** *J Clin Microbiol.* 2011; **49**(6): 2318–20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kamau E, Agoti CN, Lewa CS, et al.: **Recent sequence variation in probe binding site affected detection of respiratory syncytial virus group B by real-time RT-PCR.** *J Clin Virol.* 2017; **88**: 21–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pneumonia Etiology Research for Child Health (PERCH) Study Group: **Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study.** *Lancet.* 2019; **394**(10200): 757–79.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Otieno GP, Murunga N, Agoti C, et al.: **Replication Data for: Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya.** Harvard Dataverse. 2020.
<http://www.doi.org/10.7910/DVN/ZQ1DJY>
- Ogimi C, Englund JA, Bradford MC, et al.: **Characteristics and Outcomes of Coronavirus Infection in Children: The Role of Viral Factors and an Immunocompromised State.** *J Pediatr Infect Dis Soc.* 2019; **8**(1): 21–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gaunt ER, Hardie A, Claas ECJ, et al.: **Epidemiology and Clinical Presentations of the Four Human Coronaviruses 229E, HKU1, NL63, and OC43 Detected over 3 Years Using a Novel Multiplex Real-Time PCR Method.** *J Clin Microbiol.* 2010; **48**(8): 2940–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hoek LVD, Pyrc K, Berkhout B: **Human coronavirus NL63, a new respiratory virus.** *FEMS Microbiol Rev.* 2006; **30**(5): 760–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Aldridge RW, Lewer D, Beale S, et al.: **Seasonality and immunity to laboratory-confirmed seasonal coronaviruses (HCoV-NL63, HCoV-OC43, and HCoV-229E): results from the Flu Watch cohort study [version 1; peer review: 2 approved with reservations].** *Wellcome Open Res.* 2020; **5**: 52.
[Publisher Full Text](#)
- Kamau E, Luka MM, Laurent ZR de, et al.: **Genome Sequences of Human Coronavirus OC43 and NL63, Associated with Respiratory Infections in Kilifi, Kenya.** *Microbiol Resour Announc.* 2019; **8**(46): e00730–19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kiyuka PK, Agoti CN, Munywoki PK, et al.: **Human Coronavirus NL63 Molecular Epidemiology and Evolutionary Patterns in Rural Coastal Kenya.** *J Infect Dis.* 2018; **217**(11): 1728–39.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Killerby ME, Biggs HM, Haynes A, et al.: **Human coronavirus circulation in the United States 2014–2017.** *J Clin Virol.* 2018; **101**: 52–6.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 23 September 2020

<https://doi.org/10.21956/wellcomeopenres.17921.r40553>

© 2020 Moore D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



David P. Moore 

¹ Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa

² Department of Paediatrics and Child Health, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa

Thank you for the opportunity of reviewing the authors' revised manuscript. This is a valuable addition to the existing literature on the burden of hospitalised pneumonia in children <5 years of age that is associated with the three endemic human coronaviruses studied.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric pneumonia aetiology; paediatric infectious diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 23 September 2020

<https://doi.org/10.21956/wellcomeopenres.17921.r40552>

© 2020 Nickbakhsh S. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Sema Nickbakhsh 

MRC-University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

I am satisfied that the authors have sufficiently addressed all the concerns raised.

One minor comment: reference #8 is available in peer reviewed published form if the authors wish to update their reference list: <https://doi.org/10.1093/infdis/jiaa185>¹.

References

1. Nickbakhsh S, Ho A, Marques D, McMenamin J, et al.: Epidemiology of Seasonal Coronaviruses: Establishing the Context for the Emergence of Coronavirus Disease 2019. *The Journal of Infectious Diseases*. 2020; **222** (1): 17-25 [Publisher Full Text](#)

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Temporal and spatial dynamics of infectious diseases; epidemiology of viral respiratory infections

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 21 July 2020

<https://doi.org/10.21956/wellcomeopenres.17592.r39427>

© 2020 Nickbakhsh S. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Sema Nickbakhsh

MRC-University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

This study describes patterns of endemic human coronavirus detections over a thirteen year period among paediatric cases of pneumonia in coastal Kenya.

The authors should be commended for the impressive collation of clinical features, discharge diagnosis, and hospitalisation outcomes alongside multiplex PCR tests which is rarely available for a study spanning this length of time.

The manuscript is well written and clearly presented throughout. I do however have some concerns requiring clarification.

1. The authors mention in Methods that a variety of specimen types were collected. Could the authors clarify whether this is at the individual patient level? If relevant, I suggest authors describe how duplication of samples from individual patients was handled in the analyses

(since this can affect probability of virus detection and violates statistical assumptions of independent data points).

2. Suggest authors add to Methods a brief outline of the changes in testing procedure over time (as mentioned in the Discussion) to aid interpretation of detection trends, as different specimens are likely associated with different probabilities of virus detection.
3. It is unclear why the laboratory screen omitted HCoV-HKU1. Suggest the authors include a comment on the anticipated influence on the overall detection of HCoV.
4. It is unclear whether exclusion of untested discharged patients (presumably milder cases) have led to an under-estimation of HCoV detection proportions. Suggest authors add a statement to describe any anticipated influence of this exclusion.
5. The % of pneumonia cases with HCoV detected is clearly low. However, it would be helpful if the authors could discuss this result in the context of other common respiratory viruses detected among cases of pneumonia in this setting, such as influenza and RSV.
6. Discussion page 6: "...suggests age is not a risk factor for coronavirus associated pneumonia hospital admission". The role of age as a risk factor for pneumonia cannot be determined here without a non-pneumonia control group. Suggest authors reword this statement and consider whether the lack of variation in age-specific proportions may potentially reflect (i) no variation in age-specific community incidence of infection in children under five, together with lack of variation in severity (likelihood of hospitalisation) across ages, or (ii) variation in age-specific community incidence by age but with a disproportionate probability of hospitalisation.
7. Discussion page 9: "...and in 26% of deaths with a HCoV detected there was a likely alternative diagnosis to pneumonia". This seems to suggest the role of HCoV on overall hospital burden may be under-estimated by pneumonia cases. Although not an aim of the study, I suggest the authors consider adding a brief comment regarding the generalisability of their findings to other clinical groups to place the results into a wider context.
8. Discussion page 9: Can the authors comment on the possibility that readmissions reflect prolonged virus shedding, rather than indicating reinfection? Longest duration between admissions of 21 days possibly bordering an anticipated peak detection within first few weeks (e.g. for SARS-CoV-2¹). Suggest authors consider adding a brief statement to include this possibility.
9. Discussion page 10: "fever... was not an inclusion criterion for eligibility, which might have influenced the prevalence of HCoV". Suggest authors make clear the anticipated direction of effect on the prevalence - presumably underestimation?
10. Discussion page 10: "...there was a significant age difference for those tested and those untested for HCoV across time." This statement seems to be at odds with Results page 3 "...cases untested did not differ from those tested in age distribution.." Suggest the authors reword as appropriate.

11. Conclusions page 10: "...little evidence of a substantial aetiological contribution...": The aetiological role of HCoV cannot be established without a control group (as the authors do mention on page 9) i.e. it is not known whether the prevalence of HCoV is lower among cases of ARI without pneumonia. Suggest authors rephrase to put the low HCoV detections within the context of relative contribution of pathogens/other causes to disease aetiology i.e. most pneumonia cases are attributable to other causes.
12. Although presumably outside the scope of this study to analyse, I suggest the authors consider commenting briefly on any potential role of coinfecting respiratory bacteria on HCoV-associated pneumonia in this population; bacterial coinfection may influence the chance of pneumonia/testing for viruses.

References

1. Sethuraman N, Jeremiah SS, Ryo A: Interpreting Diagnostic Tests for SARS-CoV-2. *JAMA*. 2020. [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Temporal and spatial dynamics of infectious diseases; epidemiology of viral respiratory infections

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Sep 2020

Grievan Otieno, Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya

We wish to thank the reviewer for the detailed review of the manuscript and the suggested changes/edits. Please see below our responses:

1. The authors mention in Methods that a variety of specimen types were collected. Could the authors clarify whether this is at the individual patient level? If relevant, I suggest authors describe how duplication of samples from individual patients was handled in the analyses (since this can affect probability of virus detection and violates statistical assumptions of independent data points).

Response: We collected one sample type per person, but the type varied over time, and we have indicated this in the Methods text of the manuscript

1. Suggest authors add to Methods a brief outline of the changes in testing procedure over time (as mentioned in the Discussion) to aid interpretation of detection trends, as different specimens are likely associated with different probabilities of virus detection.

Response: *We have made the change to the methods section as suggested by the reviewer.*

1. It is unclear why the laboratory screen omitted HCoV-HKU1. Suggest the authors include a comment on the anticipated influence on the overall detection of HCoV.

Response: *We added a comment to the limitations of the study.*

1. It is unclear whether exclusion of untested discharged patients (presumably milder cases) have led to an under-estimation of HCoV detection proportions. Suggest authors add a statement to describe any anticipated influence of this exclusion.

Response: We recognise that eligible but untested individuals represent a possible bias. We state that the untested tended to be the more severe, and these are an important group, whose omission might lead to an under-estimate of the role of HCoVs in severe disease. Note however, that the prevalence of HCoV infection (any type) was less prevalent amongst those with hypoxemia (a feature of very severe pneumonia).

1. The % of pneumonia cases with HCoV detected is clearly low. However, it would be helpful if the authors could discuss this result in the context of other common respiratory viruses detected among cases of pneumonia in this setting, such as influenza and RSV.

Response: We have previously presented in several papers that relative to RSV and

rhinovirus the prevalence of all other viruses (including influenza) is low and not dissimilar to that of the HCoV - Berkley et al JAMA 2010 May 26;303(20):2051-7. doi: 10.1001/jama.2010.675 and Onyango et al J Infect Dis . 2012 Dec 15;206 Suppl 1(Suppl 1):S61-7. doi: 10.1093/infdis/jis536, and Otieno et al BMC Infect Dis . 2019 Aug 30;19(1):757.doi: 10.1186/s12879-019-4381-9

1. Discussion page 6: "...suggests age is not a risk factor for coronavirus associated pneumonia hospital admission". The role of age as a risk factor for pneumonia cannot be determined here without a non-pneumonia control group. Suggest authors reword this statement and consider whether the lack of variation in age-specific proportions may potentially reflect (i) no variation in age-specific community incidence of infection in children under five, together with lack of variation in severity (likelihood of hospitalisation) across ages, or (ii) variation in age-specific community incidence by age but with a disproportionate probability of hospitalisation.

Response: *We have made the change as suggested by the reviewer and expounded on possible role the age structure of the community plays on our findings.*

1. Discussion page 9: "...and in 26% of deaths with a HCoV detected there was a likely alternative diagnosis to pneumonia". This seems to suggest the role of HCoV on overall hospital burden may be under-estimated by pneumonia cases. Although not an aim of the study, I suggest the authors consider adding a brief comment regarding the generalisability of their findings to other clinical groups to place the results into a wider context.

Response: *We have added a statement indicating the possible occurrence of HCoV in other clinical groups in Kilifi hence potential underestimation of the proportion of deaths.*

1. Discussion page 9: Can the authors comment on the possibility that readmissions reflect prolonged virus shedding, rather than indicating reinfection? Longest duration between admissions of 21 days possibly bordering an anticipated peak detection within first few weeks (e.g. for SARS-CoV-21). Suggest authors consider adding a brief statement to include this possibility.

Response: It is possible these are prolonged infections rather than reinfections and we have added the following to the text in the discussion: "...probability of severe reinfection. Alternatively, these readmissions might be children with a prolonged infection having not fully recovered at their first discharge"

1. Discussion page 10: "fever... was not an inclusion criterion for eligibility, which might have influenced the prevalence of HCoV". Suggest authors make clear the anticipated direction of effect on the prevalence - presumably underestimation?

Response: This is possible, but we don't know that these endemic HCoVs behave like SARS-CoV-2 ie are strongly associated with fever, hence again difficult to speculate as to which direction. We therefore added this statement to the limitations "If the endemic COVs are strongly associated with symptoms of fever, as is SARS=CoV-2, then we might be under-

estimating the prevalence due to a selection bias”.

Discussion page 10: “...there was a significant age difference for those tested and those untested for HCoV across time.” This statement seems to be at odds with Results page 3 “..cases untested did not differ from those tested in age distribution..” Suggest the authors reword as appropriate.

Response: *We have made the change as suggested by the reviewer.*

Conclusions page 10: “...little evidence of a substantial aetiological contribution...”: The aetiological role of HCoV cannot be established without a control group (as the authors do mention on page 9) i.e. it is not known whether the prevalence of HCoV is lower among cases of ARI without pneumonia. Suggest authors rephrase to put the low HCoV detections within the context of relative contribution of pathogens/other causes to disease aetiology i.e. most pneumonia cases are attributable to other causes.

Response: *We have made the change as suggested by the reviewer.*

Although presumably outside the scope of this study to analyse, I suggest the authors consider commenting briefly on any potential role of coinfecting respiratory bacteria on HCoV-associated pneumonia in this population; bacterial coinfection may influence the chance of pneumonia/testing for viruses.

Response: We had very low proportions of bacterial infections among the positive cases: NL63 (2/74=2.7%), OC43 (3/129=2.3%), 229E (2/99=2.02%). We feel there is too little co-infection to allow speculation on this.

Competing Interests: No competing interests declared

Reviewer Report 06 July 2020

<https://doi.org/10.21956/wellcomeopenres.17592.r39270>

© 2020 Moore D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



David P. Moore

¹ Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa

² Department of Paediatrics and Child Health, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa

Thank you for the opportunity of reviewing this well-written manuscript by the accomplished research team at Kilifi. They describe the prevalence of endemic human coronavirus (HCoV)

pneumonia hospitalisations in children <5 years of age in Kilifi over a 13-year surveillance period.

Major comments:

1. Why was HCoV-HKU1 not included in the surveillance of endemic human coronaviruses in this study? Lack of testing for HCoV-HKU1 should be mentioned as a limitation of the study.
 - PERCH samples were included in the analysis, which would have included HCoV-HCoV-HKU1: mention should be made that, as HKU1 was only tested for in the 24-month PERCH surveillance period, it was considered to be under-represented over the study time period and was therefore not included in the analysis.
2. Mention is made of a variety of specimen types that were tested for respiratory viruses in the study. Is there evidence to suggest that any one type gives better yields than others? This should be elaborated on briefly in the discussion section, potentially as a limitation of the study.
3. The authors mention that an alternative attribution could be made in 26% of cases that died with a positive detection of HCoV in their study (page 9, left hand column, third sentence); however, this is not explored at all in the Results Section. This should be presented in the Results section, so as to contextualise the assertion made in the Discussion.

Minor comments:

1. Suggest change the title to reflect that paediatric pneumonia was the focus of attention in this study. Suggest "Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with childhood pneumonia in Kilifi, Kenya".
2. Correct the redundant double comma in affiliation 2 at the top of the title page of the manuscript.
3. In the results section of the Abstract, qualify that 3.9% of the pneumonia admissions tested positive for at least one of the three endemic HCoVs surveyed in the study.
4. It is unclear what is meant by "no age-dependence in the proportion testing positive" for HCoV in the abstract. Suggest omit this ambiguous phrase. Rather state "... n=208), and there was no clear seasonal pattern of HCoV infection. HCoV-OC43 was predominant..."
5. Suggest reword the Abstract conclusions as "... co-occurring with another respiratory virus, no clear seasonal pattern, and with the age-distribution..."
6. Suggest omit the speculation as to the impact of SARS-CoV-2 at the end of the last sentence of the Abstract.
7. At the end of the Introduction, suggest reword as "Our study aims to describe the circulation patterns of three of the endemic HCoVs (OC43, 229E, and NL63) over time..."
8. Suggest omit the definite article in the first sentence of the Methods section, i.e. "... in 2007 for long-term continuous respiratory virus surveillance among..."
9. Consider amending slight punctuation errors in page 3, including:
 - Left column, six lines from the bottom: "... $\geq 37.5^{\circ}\text{C}$, oxygen..."

- Right column, second line: "... alive or dead)."
10. There are two mentions of which viruses were tested for on page 3: suggest concatenate into one mention (probably best placed in the "Laboratory methods" paragraph). Reword at the bottom of page 3 on the right hand column to "... or very severe), laboratory test results, and hospitalisation outcomes..."
 11. The link to code in *Extended data* is not currently active in the manuscript: please correct this.
 12. At the bottom of page 3, suggest reword as "Untested cases did not differ... more likely to have very severe pneumonia (40.0% versus 28.3%; $\chi^2=152.5$, $P<0.001$)."
 - Why resort to the use of Fisher's exact P-value in the analysis of tested and untested cases (the Chi-square P-value is every bit as "significant")?
 13. Children are not "samples": suggest reword the sentence at the top of page 4 as "Of the 7957 children tested, 5312 (66.7%) were aged <1 year, 1454..."
 14. Suggest clarify the meaning of the second sentence on page 4 as follows: "The proportion of tested individuals with fever (axillary temperature $\geq 37.5^\circ\text{C}$), cough, and difficulty breathing was 58.3%, 83.2% and 92.6%, respectively."
 15. On page 4, suggest reword the third last line on the left hand column as "... testing positive for HCoV (3.9%) did not vary with age group..."
 16. In Table 1, suggest present Age (months) as median and interquartile range only.
 17. In Table 1, rather use the term "Difficulty breathing" which is more widely used than "Breathing difficulty", also use "Prostration" rather than "Prostrate/unconscious".
 18. There are typos in the footnote to Table 1: "tde" should be "the"; "breatding difficulty" should be "difficulty breathing"; "witd" should be "with".
 19. Suggest reword the first sentence under the heading "Clinical outcomes of HCoV-infected patients" on page 5 as: "... positive for at least one of the three endemic HCoVs investigated for in this study were admitted to..."
 20. In Figure 1, the HCoV-OC43 figure y-axis requires correction of spelling of the word "immunosuppression"; the HCoV-229E figure requires change of the "Malnutrition & immunosuppression" label on the y-axis to have an initial capital "M".
 21. Suggest reword the first sentence under the heading "Temporal patterns of different HCoVs" on page 5 as: "... middle of 2011, and were not detected subsequent to 2016 (Figure 3)."
 22. In Table 2, suggest add in an analysis for all HCoVs combined.
 23. Suggest reword the first sentence of the Discussion as "... patterns of three endemic HCoVs (NL63, OC43 and 229E) in a... positive for one or more of the HCoVs that were investigated for (3.9%)."

24. Check the calculation of the comparison between Sipulwa et al and the current study, in the second line on the right hand column on page 9: I get $X^2=3.92$, $p=0.048$.
25. In the second sentence of the second paragraph on the right column on page 9, suggest reword to "... nor the long rainy periods (April to July) in Kilifi were associated with HCoV peaks."
26. Suggest reword the fifth sentence in the right hand column on page 9 as "Of interest is that pneumonia associated with 229E was not detected in the later years of surveillance."
27. Please correct the spelling or "axillary" in the footnote to Table 2.
28. The authors do not consider differences in participant recruitment as being a potential explanation as to why 229E-associated pneumonia was not detected subsequent to 2016: were there systematic differences in participant recruitment strategies that might have impacted on detection of this virus?
29. Suggest reword the third sentence on the right hand column on page 10 as "It should be noted that fever, either on history or as measured at the time of admission, was not an inclusion criterion..."

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric pneumonia aetiology; paediatric infectious diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Sep 2020

Grievan Otieno, Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya

Major comments:

1. Why was HCoV-HKU1 not included in the surveillance of endemic human coronaviruses in this study? Lack of testing for HCoV-HKU1 should be mentioned as a limitation of the study. PERCH samples were included in the analysis, which would have included HCoV-HCoV-HKU1: mention should be made that, as HKU1 was only tested for in the 24-month PERCH surveillance period, it was considered to be under-represented over the study time period and was therefore not included in the analysis.

Response: *We thank the reviewer for pointing this out. We have edited the manuscript as advised by the reviewer.*

1. Mention is made of a variety of specimen types that were tested for respiratory viruses in the study. Is there evidence to suggest that any one type gives better yields than others? This should be elaborated on briefly in the discussion section, potentially as a limitation of the study.

Response: *We thank the reviewer for pointing this out. We have added the following statement to the limitations of the study*
"We employed various collection methods and these are known to differ in the range of viruses detected. For example, the addition of an OP swab to an NP swab has been found to increase virus yield"

1. The authors mention that an alternative attribution could be made in 26% of cases that died with a positive detection of HCoV in their study (page 9, left hand column, third sentence); however, this in not explored at all in the Results Section. This should be presented in the Results section, so as to contextualise the assertion made in the Discussion.

Response: *We have expanded the statements in the results section to refer to the proportion of individuals and made adjustments to the discussion section.*

Minor comments:

1. Suggest change the title to reflect that paediatric pneumonia was the focus of attention in this study. Suggest "Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with childhood pneumonia in Kilifi, Kenya".

Response: *We have made the change as suggested by the reviewer.*

1. Correct the redundant double comma in affiliation 2 at the top of the title page of the

manuscript.

Response: *We have made the change as suggested by the reviewer.*

1. In the results section of the Abstract, qualify that 3.9% of the pneumonia admissions tested positive for at least one of the three endemic HCoVs surveyed in the study.

Response: *We have made the change as suggested by the reviewer.*

1. It is unclear what is meant by “no age-dependence in the proportion testing positive” for HCoV in the abstract. Suggest omit this ambiguous phrase. Rather state “... n=208), and there was no clear seasonal pattern of HCoV infection. HCoV-OC43 was predominant...”

Response: We have adjusted the statement in the abstract but would wish to retain the rest. Although there were more cases in the younger age groups this simply reflected the more eligible admissions.

1. Suggest reword the Abstract conclusions as “... co-occurring with another respiratory virus, no clear seasonal pattern, and with the age-distribution...”

Response: *We have made the change as suggested by the reviewer.*

1. Suggest omit the speculation as to the impact of SARS-CoV-2 at the end of the last sentence of the Abstract.

Response: *We have made the change as suggested by the reviewer.*

1. At the end of the Introduction, suggest reword as “Our study aims to describe the circulation patterns of three of the endemic HCoVs (OC43, 229E, and NL63) over time...”

Response: *We have made the change as suggested by the reviewer.*

1. Suggest omit the definite article in the first sentence of the Methods section, i.e. “... in 2007 for long-term continuous respiratory virus surveillance among...”

Response: *We have made the change as suggested by the reviewer.*

1. Consider amending slight punctuation errors in page 3, including: Left column, six lines from the bottom: “... $\geq 37.5^{\circ}\text{C}$, oxygen...”

Right column, second line: "... alive or dead)."

Response: *We have made the change as suggested by the reviewer.*

1. There are two mentions of which viruses were tested for on page 3: suggest concatenate into one mention (probably best placed in the "Laboratory methods" paragraph). Reword at the bottom of page 3 on the right hand column to "... or very severe), laboratory test results, and hospitalisation outcomes..."

Response: *We have made the change as suggested by the reviewer.*

1. The link to code in Extended data is not currently active in the manuscript: please correct this.

Response: *We have corrected the error with the link and it is now active.*

1. At the bottom of page 3, suggest reword as "Untested cases did not differ... more likely to have very severe pneumonia (40.0% versus 28.3%; $X = 152.5$, $P < 0.001$). Why resort to the use of Fisher's exact P-value in the analysis of tested and untested cases (the Chi-square P-value is every bit as "significant")?

Response: *Fisher's exact p-value is reported because of the low expected counts (<5) in the older age bands*

1. Children are not "samples": suggest reword the sentence at the top of page 4 as "Of the 7957 children tested, 5312 (66.7%) were aged <1 year, 1454..."

Response: *We have made the change as suggested by the reviewer.*

1. Suggest clarify the meaning of the second sentence on page 4 as follows: "The proportion of tested individuals with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$), cough, and difficulty breathing was 58.3%, 83.2% and 92.6%, respectively."

Response: *We have made the change as suggested by the reviewer.*

1. On page 4, suggest reword the third last line on the left hand column as "... testing positive for HCoV (3.9%) did not vary with age group..."

Response: *We have made the change as suggested by the reviewer.*

1. In Table 1, suggest present Age (months) as median and interquartile range only.

Response: *We have made the change as suggested by the reviewer.*

1. In Table 1, rather use the term "Difficulty breathing" which is more widely used than "Breathing difficulty", also use "Prostration" rather than "Prostrate/unconscious".

Response: *We have made the change as suggested by the reviewer.*

1. There are typos in the footnote to Table 1: "tde" should be "the"; "breatding difficulty" should be "difficulty breathing"; "witd" should be "with".

Response: *We have made the change as suggested by the reviewer.*

1. Suggest reword the first sentence under the heading "Clinical outcomes of HCoV-infected patients" on page 5 as: "... positive for at least one of the three endemic HCoVs investigated for in this study were admitted to..."

Response: *We have made the change as suggested by the reviewer.*

1. In Figure 1, the HCoV-OC43 figure y-axis requires correction of spelling of the word "immunosuppression"; the HCoV-229E figure requires change of the "Malnutrition & immunosuppression" label on the y-axis to have an initial capital "M".

Response: *We have made the change as suggested by the reviewer.*

1. Suggest reword the first sentence under the heading "Temporal patterns of different HCoVs" on page 5 as: "... middle of 2011, and were not detected subsequent to 2016 (Figure 3)."

Response: *We have made the change as suggested by the reviewer.*

1. In Table 2, suggest add in an analysis for all HCoVs combined.

Response: *We have made the change as suggested by the reviewer.*

1. Suggest reword the first sentence of the Discussion as "... patterns of three endemic HCoVs (NL63, OC43 and 229E) in a... positive for one or more of the HCoVs that were investigated for (3.9%)."

Response: *We have made the change as suggested by the reviewer.*

1. Check the calculation of the comparison between Sipulwa et al and the current study, in the second line on the right hand column on page 9: I get $X = 3.92$, $p = 0.048$.

Response: *We have made the change as suggested by the reviewer.*

1. In the second sentence of the second paragraph on the right column on page 9, suggest reword to "... nor the long rainy periods (April to July) in Kilifi were associated with HCoV peaks."

Response: *We have made the change as suggested by the reviewer.*

1. Suggest reword the fifth sentence in the right hand column on page 9 as "Of interest is that pneumonia associated with 229E was not detected in the later years of surveillance."

Response: *We have made the change as suggested by the reviewer.*

1. Please correct the spelling or "axillary" in the footnote to Table 2.

Response: *We have made the change as suggested by the reviewer.*

1. The authors do not consider differences in participant recruitment as being a potential explanation as to why 229E-associated pneumonia was not detected subsequent to 2016: were there systematic differences in participant recruitment strategies that might have impacted on detection of this virus?

Response: *We have made the change as suggested by the reviewer.*

1. Suggest reword the third sentence on the right hand column on page 10 as "It should be noted that fever, either on history or as measured at the time of admission, was not an inclusion criterion..."

Response: *We have made the change as suggested by the reviewer.*

Competing Interests: No competing interests declared